Comparison of the prevalence of chronic endometritis as determined by means of different diagnostic methods in women with and without reproductive failure

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Objective: To compare the prevalence of chronic endometritis (CE) when different diagnostic methods are used.
Design: Prospective observational study.
Setting: University-affiliated hospital.
Patient(s): Four groups of women were studied, including women with proven fertility (Fertile; n = 40), unexplained recurrent miscarriage (RM; n = 93), recurrent implantation failure (RIF; n = 39), and infertile subjects undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer (Infertility; n = 48).
Intervention(s): Endometrial biopsy was performed precisely 7 days after LH surge (LH+7). Plasma cells were identified by means of traditional hematoxylin and eosin (HE) staining and by means of immunohistochemistry (IHC) for Syndecan-1 (CD138).
Main Outcome Measure(s): Prevalence of CE.
Result(s): The use of CD138 epitope was more sensitive than HE staining in identifying plasma cells. The use of plasma cell count per unit area had the lowest observer variability compared with cell count per ten randomly chosen high-power fields and cell count per section. Using this method, the prevalence of CE in women with RM, RIF, and Infertility were 10.8%, 7.7%, and 10.4%, respectively, not significantly higher than that of Fertile subjects (5.0%).
Conclusion(s): Using what may be a new method of plasma cell assessment, it appears that the prevalence rates of CE reported in many earlier studies may have been overestimated.
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Key Words: Chronic endometritis, plasma cell, prevalence, reference range, reproductive failure

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Chronic endometritis (CE) refers to local persistent inflammation of the endometrium. CE has been reported to be associated with various subgroups of reproductive failure, including infertility (1–3), recurrent miscarriage (RM) (4–8), and recurrent implantation failure (RIF) (4,9–11).

The presence of plasma cells in endometrial stroma has been accepted as the criterion standard method to establish a diagnosis of CE (12).

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Nevertheless, the reported prevalence of CE in endometrial biopsy specimens has varied considerably, ranging from 3% to 60% (Table 1). There are several possible explanations to account for the wide variation reported. First, there are two different methods used to identify plasma cells. Traditionally, plasma cells are identified in hematoxylin and eosin (HE)–stained specimens. However, the identification of plasma cells in HE sections requires experience coupled with diligent search, without which they can be easily missed. A more recently introduced method is immunohistochemistry (IHC) staining for Syndecan-1 (CD138), a proteoglycan found on the cell surface of plasma cells and keratinocytes. This has been found to improve the sensitivity and accuracy for identifying the plasma cells essential for the diagnosis of CE (13–15).

Second, various investigators have used different approaches to quantify the CD138+ cell count (Table 1). In the first approach, the number of plasma cell per whole section was measured. In the second approach, the plasma cell count per a defined number of (e.g., ten) randomly chosen high-power fields (HPFs) was measured. There are rationales behind each of these two approaches. Some investigators have advocated scrutinizing the entire specimen because they thought that plasma cells are not normally present in the endometrium and the finding of one or more plasma cells is indicative of a diagnosis of CE (12, 16, 17). One shortcoming of such an approach is that it does not take into account the size of the specimen. One would expect that, other things being equal, the larger the specimen size, the more likely it is to find plasma cells, and vice versa. Consequently, other authors introduced the concept of plasma cell density to correct for the size of the specimen examined; they advocated examining ten or more chosen HPFs and expressing the number of plasma cells detected per HPF or per ten HPFs, because each HPF is equivalent to a defined area (4, 6, 10, 11, 15, 18, 19). To avoid bias in selecting the HPFs to be examined and to improve objectivity, it is desirable to have randomly chosen fields. However, the potential disadvantage of such an approach is that plasma cells are usually present in low numbers, so the inclusion of only ten selected HPFs may not be sufficient to produce a consistently reproducible result. We postulate that a new method of plasma cell assessment that combines the positive attributes of the two above-mentioned methods would be to count all CD138+ cells in the entire section, then measure the area of the examined tissue section and express the result as plasma cell count per unit area. In this way, it would overcome the problem of local fluctuation of plasma cell count as well as correcting for the variation in results due to sample size difference.

There is also no consensus on the diagnostic criteria used to define what constitutes CE. At least seven criteria have been reported in the literature, including at least one plasma cell per section (20), at least one plasma cell per HPF (10), at least one plasma cell per ten HPFs (3), at least five plasma cells per ten HPFs (4), at least five plasma cells per 20 HPFs (22), the presence of one to five plasma cells per HPF or discrete clusters of <20 plasma cells (7), and an endometrial stromal plasmacyte density index (the sum of the stromal CD138+ cell counts divided by the number of the HPFs evaluated) of ≥0.25 (11) (Table 1). The proposed criteria are all rather arbitrarily chosen and not based on reference ranges derived from normal fertile populations.

In the present study, our aim was to establish a reference range of plasma cell count in the endometrium of fertile subjects with the use of two different methods of identification and three different methods of quantification, as discussed above, followed by a comparison of the performance of these methods. The prevalence rates of CE so derived among women with reproductive failure was then determined, using this methodology, with a view to determining the optimal strategy to identify and quantify plasma cells and to diagnose CE.

TABLE 1

Prevalence of chronic endometritis reported in the literature among three groups of women (infertility, recurrent miscarriage, and recurrent implantation failure) in relation to inclusion criteria, diagnostic criteria, and timing of endometrial biopsy.

Reference	Inclusion criteria	Diagnostic criteria (plasma cell count)	Timing of endometrial biopsy	Prevalence
Infertility				
Cicinelli et al., 2005	Unexplained infertility	≥1/section	Follicular phase	30% (45/150)
Kitaya and Yasuo, 2010	Unexplained infertility	≥1/10 HPFs	LH+6–8	29% (22/76)
Kasius et al., 2011	Infertility	≥1/section	Follicular phase	3% (17/606)
Kitaya et al., 2012	Infertility	≥5/20 HPFs	Follicular phase	44% (23/52)
Recurrent miscarriage				
Kitaya, 2011	≥3 miscarriages	≥1/10 HPFs	LH+6–8	9% (5/54)
Zolghadri et al., 2011	≥3 miscarriages	≥1/section	Follicular phase	43% (61/142)
Cicinelli et al., 2014	≥3 miscarriages	≥1/section	Follicular phase	53% (190/360)
McQueen et al., 2015	≥2 miscarriages	1–5/HPF or discrete clusters <20	Not mentioned	56% (60/107)
Bouet et al., 2016	≥2 unexplained miscarriages	≥5/10 HPFs	Follicular phase	27% (14/51)
Recurrent implantation failure				
Johnston-MacAnanny et al., 2010	≥2 failed ET cycles or >10 failed ETs	≥1/HPF	Not mentioned	30% (10/33)
Kitaya et al., 2017	≥3 failed ETs	ESPDI ≥0.25	Follicular phase	34% (142/421)
Cicinelli et al., 2015	≥3 failed ET cycles	≥1/section	Follicular phase	57% (61/106)
Bouet et al., 2016	≥3 failed ETs	≥5/10 HPFs	Follicular phase	14% (6/43)

Note: ESPDI = endometrial stromal plasmacyte density index; ET = embryo transfer; HPF = high-power field, ×400 magnification; LH+6–8 = 6 to 8 days after LH surge.

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MATERIALS AND METHODS

Participants

Subjects were recruited from women attending the Department of Obstetrics and Gynecology, Prince of Wales Hospital, The Chinese University of Hong Kong. Women were recruited from four groups: 1) fertile control group: healthy women with at least one live birth within the previous 2 years (n = 40); 2) unexplained RM group: women with the loss of three or more consecutive pregnancies before 24 weeks of gestation (23) (n = 93); 3) RIF group: women younger than 40 years of age failing to achieve a clinical pregnancy after transfer of at least four good-quality embryos in three or more transfer cycles (24) (n = 39); and 4) infertile group: women with infertility undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer with the use of nondonor oocytes (n = 48).

The inclusion criteria were women 20–40 years of age with regular menstrual cycle (25–35 days), with normal pelvic ultrasonography, and who had not used any antibiotics, estrogen or progestogen hormonal therapy, steroid treatment, or intrauterine contraceptive device within 2 months before recruitment. The exclusion criteria included the presence of hydrosalpinx, structural uterine abnormalities, parental chromosomal abnormalities, and significant medical conditions such as systemic lupus erythematosus.

Endometrial Biopsy

All subjects in this study had a daily urine dipstick test from day 9 of the menstrual cycle onward to identify the LH surge (ovulation), which was used to precisely time the endometrial biopsies to 7 days after the LH surge (day LH+7). All biopsies were obtained with the use of a Pipelle sampler (Prodimed) or Pipet Curettage (Cooper Surgical). The specimens were immediately placed into 10% neutral buffered formalin for overnight fixation at room temperature and then embedded into paraffin wax.

Processing of Specimen

The paraffin-embedded human endometrial tissues were cut into sections (4 μm), dewaxed in xylene, and rehydrated through descending ethanol to phosphate-buffered saline solution (PBS).

Hematoxylin-eosin staining. Paraffin-embedded human endometrial tissue sections were examined with the use of routine HE staining.

Immunohistochemistry staining. Slides were pretreated with microwave heating for 20 minutes in sodium citrate buffer for antigen retrieval, and then quenched with 0.3% v/v hydrogen peroxidase in methanol to block endogenous peroxidase activity. Then the sections were blocked with the use of rabbit serum to prevent nonspecific binding and then incubated with a 1:50 dilution of mouse monoclonal antibody against human Syndecan-1 (clone B-A38; Cell Marque) overnight at 4°C. After incubation, the sections were washed in PBS–Tween 20 and incubated with secondary rabbit antimouse horseradish peroxidase–labeled antibody (1:100, ab97046; Abcam) for 1 hour, followed by color development with 3,3-diaminobenzidine (Dako), counterstained with hematoxylin, dehydrated with ethanol, cleared in xylene, and mounted with a cover slide.

Image Acquisition and Analysis

Image analysis was performed by one operator who first scanned the slides at lower magnification, and then captured images (×400) of all the fields of CD138+ plasma cells with the use of the Leica DM6000B system. Then the whole section of each sample was tile scanned under ×50 magnifications by the same system, which was able to merge separate images into one image covering all of the tissue. Cell counts and section area were analyzed with the use of Image J (version 1.51a; Wayne Rasband, National Institutes of Health). The total number of plasma cells was determined by counting immune-positive cells in the entire specimen. Cells were considered to be likely CD138+ plasma cells if they exhibited unambiguous complete brown staining with intact cell membrane, a clearly defined nucleus typical of a plasma cell, and occurred singly or as small clusters of cells, excluding background stroma, glands, and other confounders. The identification and counting of the CD138+ cells was performed manually under the microscope, whereas the measurement of the specimen area was made by Image software on images captured in the computer.

Determination of Observer Variability

The intra-observer variability of CD138+ cell count was determined by asking a single observer to repeat the measurement of 20 randomly chosen specimens on two separate occasions, without knowledge of the results of the first count. The observer used three different quantification methods to do the measurement, namely method I) CD138+ cell count per ten randomly chosen HPFs; method II) CD138+ cell count per whole section; and method III) CD138+ cell count per unit area (cell density). For the third method, Image J software was used to measure the area of the tissue section.

The interobserver variability was determined by asking two observers (Y.L. and X.C.) to perform the measurement of CD138+ cell count on the same set of 20 randomly chosen specimens independently from each other, also using the three different quantification methods as in the case of intra-observer variability study.

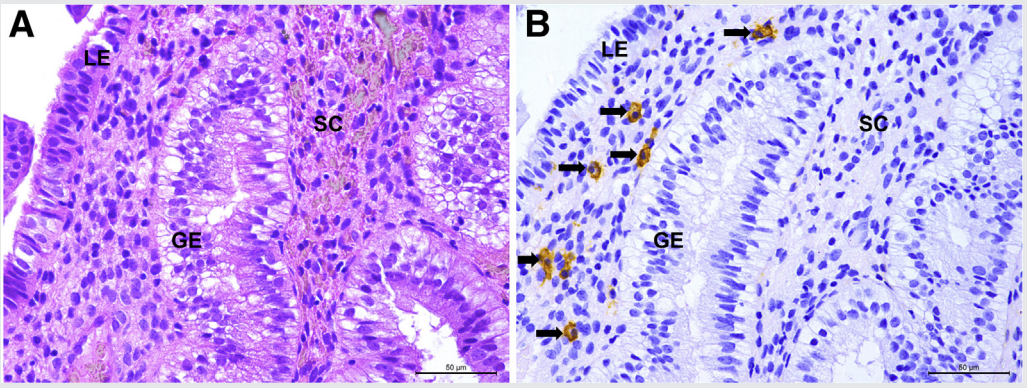
Reference Range

In this study, the reference range of plasma cell count or density was derived from the 40 fertile control subjects. Values below the 95th percentile were considered to be normal, whereas values above the 95th percentile were considered to be abnormal and indicative of a diagnosis of CE.

Definition of CE

Chronic endometritis was defined as the presence of CD138+ plasma cell count or density above the established reference

FIGURE 1



Expression of plasma cells using (**A**) hematoxylin and eosin staining and (**B**) immunohistochemistry staining for syndecan-1 (CD138) in the same field of endometrial tissue from the same woman. Plasma cells are indicated by *arrows*. GE = glandular epithelium; LE = luminal epithelium; SC = stromal cell. Magnification = ×400; scale bar = 50 μm.

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range (95th percentile) in whatever quantification method was used.

Ethical Considerations

This study was approved by the local hospital Ethics Committee (CREC ref. no. 2015.477). Written informed consent was obtained from all participants.

Statistical Analysis

After analyzing the distribution of our data and confirming that results in the control population were not normally distributed, we adopted a nonparametric method to analyze the data and used 95th percentile as the cutoff instead of 2 SD above the mean (parametric method) as the cutoff. The maternal age and body mass index (BMI) of the women in the four groups were compared by analysis of variance. Intra- and interclass correlation coefficients (intra- and inter-CC) with 95% confidence intervals (CIs) were used to assess the intra- and interobserver agreements in the calculation of plasma cell count, area of tissue examined, and plasma cell density (intra- and inter-CC values <0.40 were considered to represent poor, 0.40–0.75 moderate, and >0.75 excellent agreement). Chi-square test was used to compare the prevalence of CE between subgroups. Statistical analysis was performed with the use of SPSS version 23.0, and a P value of $<.05$ was considered to be represent statistical significance.

RESULTS

From December 2014 to June 2017, 229 subjects underwent endometrial biopsy. Nine subjects were excluded because of insufficient tissue obtained. In total, 220 subjects were included in the study.

Demographics

The demographic details of the subjects are summarized in Supplemental Table 1 (available online at www.fertstert.org). The mean age of all of the subjects was 34.4 (range 21–40) years. The mean age of reproductive failure group (35.4 ± 3.1 years) was significantly higher ($P<.01$) than that of Fertile control group (29.6 ± 3.4 years); there was no significant difference among the RM, RIF, and Infertility subgroups. There was also no significant difference in body mass index between groups.

Identification of Plasma Cells

No classic plasma cells were identified in any of the routine HE-stained sections (n = 220) examined in this study, whereas the use of CD138 IHC staining identified the presence of one or more plasma cells in 95 (43.2%) of the specimens (Fig. 1). In most cases, the distribution of plasma cells within the endometrium was not uniform, being localized focally or widely dispersed in the stroma.

Intra-observer Variability of Quantification Methods

The results of the intra-observer variability of the three different quantification methods of measurement (method I: CD138+ cell count per ten randomly chosen HPFs; method II: CD138+ cell count per whole section; and method III: CD138+ cell count per unit area (cell density) are compared in Supplemental Table 2 (available online at www.fertstert.org). The intra-CC value of cell count per ten randomly chosen HPFs was 0.46, which was considered to be moderate. The intra-CCs of cell count per whole section and cell count per unit area of whole section were 0.90 and 0.84, respectively, both considered to be excellent.

Interobserver Variability of Quantification Methods

The results of the interobserver variability of the three different methods of measurement are compared in Supplemental Table 3 (available online at www.fertstert.org).

The inter-CC of plasma cell count per ten randomly chosen HPFs was 0.39, which was considered to be poor. The inter-CCs of cell count per whole section and cell count per unit area of whole section were 0.88 and 0.83, respectively, both considered to be excellent.

Reference Range

In establishing the reference range of the plasma cell count or density, the specimens from fertile control subjects were examined and the 95th percentile of the results was used to define the upper limit of the reference range. The reference ranges for three different methods of quantification were: I) 1.95 CD138+ cells per ten randomly chosen HPFs; II) 2.95 CD138+ cells per section; and III) 5.15 CD138+ cells per 0.1 mm^2 (Table 2, Fig. 2).

Prevalence of CE

The prevalences of CE in the various subgroups in this study as determined by the three different methods of CD138+ cell quantification, in conjunction with two different diagnostic criteria (one based on a previous literature report and the other based on a reference range derived from fertile women) are compared in Table 2. Quantification using method I and method II consistently produced higher prevalence rates than quantification using method III. The application of previously published criteria consistently produced higher prevalence rates than criteria based on a reference range derived from fertile population. The prevalence of CE in women with reproductive failure as determined by quantification methods I or II, regardless of the diagnostic criteria used, was significantly higher than fertile subjects in three out of the four criteria used (Table 2). However, the prevalence of CE in women with reproductive failure and its subgroups, determined with the use of quantification method III and diagnostic criteria based on the established reference range, was not significantly ($P>.05$) higher than that of fertile subjects.

Confounding Variable

The possible impact of age on the results of the expression of CD138 was examined by means of regression analysis. There was no significant association between CD138+ cell density and age.

DISCUSSION

In this prospective observational study, we used different methods to identify and quantify plasma cell counts and applied different criteria to diagnose CE; with the use of our proposed new methods of plasma cell assessment, we found that the prevalence rates of CE reported in earlier studies of women with reproductive failure may have been overestimated.

Identification of Plasma Cells

The identification of plasma cells in endometrial biopsy specimens continues to be considered to be the criterion-standard method for the diagnosis of CE (12, 13, 25). Typical plasma

TABLE 2

The prevalence of chronic endometritis according to three different quantification methods of CD138+ cells (I, CD138+ cell count per ten randomly chosen HPFs; II, CD138+ cell count per whole section; and III, CD138+ cell count per unit area) in conjunction with selected diagnostic criteria.

	(I) CD138+ cell count per 10 randomly chosen HPF			(II) CD138+ cell count per whole section			(III) CD138+ cell count per unit area	
Population	(Ia)[a] CE+ if ≥1 cell/10 HPFs	(Ib)[b] CE+ if ≥1.95 cells/10 HPFs	*P* value[c]	(IIa)[a] CE+ if ≥1 cell/section	(IIb)[b] CE+ if ≥2.95 cells/section	*P* value[c]	(IIIb)[b] CE+ if ≥5.15 cells/0.1 mm^2	*P* value[d]
Fertile (n = 40)	17.5%	5%	.16	30.0%	5%	<.01	5%	Ib vs. IIb: 1.00 Ib vs. IIIb: 1.00 IIb vs. IIIb: 1.00
Reproductive failure (n = 180)	28.9%	18.9%	.02	46.1%	22.2%	<.01	10.0%	Ib vs. IIb: .26 Ib vs. IIIb: 0.01 IIb vs. IIIb: <.01
P value[e]	.10	.02		<.05	<.01		.49	
RM (n = 93)	19.4%	12.9%	.16	38.7%	17.2%	<.01	10.8%	Ib vs. IIb: 0.27
P value[e]	.51	.29		.22	<.05		.46	Ib vs. IIIb: 0.41 IIb vs. IIIb: 0.15
RIF (n = 39)	23.1%	15.4%	.28	51.3%	20.5%	<.01	7.7%	Ib vs. IIb: 0.38
P value[e]	.37	.25		<.05	.04		.98	Ib vs. IIIb: 0.24 IIb vs. IIIb: <1.00
Infertility (n = 48)	37.5%	29.2%	.26	56.3%	33.3%	.02	10.4%	Ib vs. IIb: 0.41 Ib vs. IIIb: 0.02
P value[e]	.03	<.01		.01	<.01		.59	IIb vs. IIIb: <.01

Note: Chi-square test was used to compare the difference between subgroups. HPF = high-power field, ×400 magnification; RIF = recurrent implantation failure; RM = recurrent miscarriage.
[a] Arbitrary criterion used in previous literature.
[b] Criterion based on reference range (95th percentile) derived from fertile subjects.
[c] Comparison of CE prevalence according to two different diagnostic criteria, a and b, with the use of the same quantification method (Ia vs. Ib and IIa vs. IIb).
[d] Comparison of CE prevalence according to three different quantification methods using the same diagnostic criteria based on reference ranges derived from fertile subjects (Ib, IIb, and IIIb).
[e] Comparison of CE prevalence according to the same quantification method and diagnostic criteria between fertile subjects and women with reproductive failure and subgroups.

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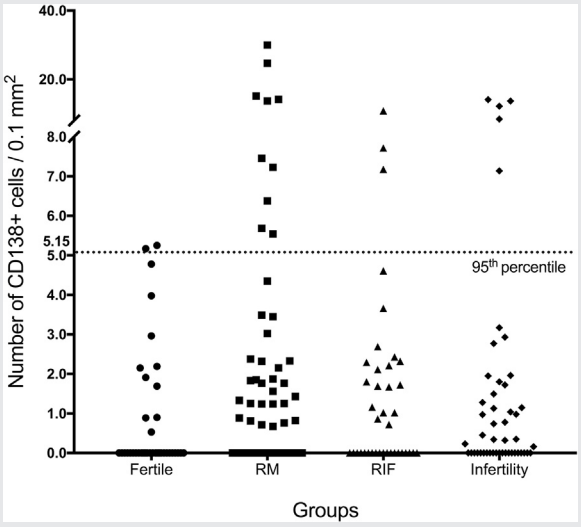
FIGURE 2



Scatter plot of CD138+ cell count per unit area (cell density) in four subgroups of women (Fertile: fertile control women; RM: unexplained recurrent miscarriage; RIF: recurrent implantation failure; Infertility: infertile women undergoing endometrial scratch in a natural cycle preceding frozen-thawed embryo transfer). Reference range of CD138+ cell count per unit area is up to 5.15 CD138+ cells/0.1 mm^2, shown on the *y* axis. There was no statistically significant difference between subgroups according to chi-square test.

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cells have a large cell body, high nuclei-cytoplasm ratio, basophilic cytoplasm, and nuclei with heterochromatin in a unique arrangement called a "spoke wheel" or "clockface" pattern (13, 17). However, plasma cells may from time to time be missed in routine histologic examination. The results from the present study agree with earlier reports (1,13–15) on the usefulness of CD138 immunostaining in the identification of plasma cells, which has been shown to be a more sensitive and accurate method to identify plasma cells compared with the conventional HE-staining method (13–15). In accordance with previous publications, we could not find any classic plasma cells in any of the HE-stained specimens, whereas the use of CD138 staining led to the detection of one or more plasma cells in 46.1% (83/180) of samples from women with reproductive failure and in 43.2% (95/220) of samples from all of the subjects in this study.

Quantification of Plasma Cell

In this study, we based the diagnosis of CE on the stromal plasma cell count only; there are a number of morphologic features that have been reported to be associated with chronic endometritis, namely, superficial stroma edema, stromal inflammatory infiltrate, increased stromal density, focal stromal hemorrhage, and spindling of stroma, most notably in the upper half of the mucosa (12). Greenwood and Morgan (12) argued for the inclusion of these additional morphologic features in the definition of CE, which was supported by Bayer-Garner and Korourian (13) and Cicinelli et al. (32), but some investigators based the diagnosis on plasma cell count only (19, 27). We did not include the additional morphologic features proposed by Greenwood and Morgan in our analysis, partly because there is as yet no consensus on the diagnostic value of these features and furthermore they are not easily quantifiable.

It should be noted that the proposed "plasma cell density" measurement in the present study referred to plasma cell count per unit area, calculated from the entire area of the specimen, consisting of all fields whether complete or not, which is different from the endometrial stromal plasmacyte density index which was calculated as the sum of the stromal CD138+ cell counts divided by the number of HPFs evaluated (11), which is in essence plasma count per selected number of complete HPFs.

Observer Variability

Observer variability is a measure of how reproducible the results are. In this study we directly compared the observer variability of three different quantification methods and found that the coefficient of variation of results obtained from the cell count per HPF method was considerably higher than that of the cell count per section method and the cell density method. It is likely that the source of variation of the cell count per HPF method comes from the random nature of selection of the HPF, primarily because the plasma cell count is low. Missing one or two positively stained cells could make a significant difference to the results. The other two methods both had intra-observer and interobserver CCs in the excellent category. The intra- and interobserver variabilities for cell density was slightly higher than for cell count per section, which is to be expected because the measurement of cell density requires an additional measurement of area and so introduces an additional source of variation.

Reference Range

In this study we established normal ranges derived from a fertile population and defined results above the 95th percentile as abnormal (and the basis for the diagnosis of CE). This approach is commonly used for establishing clinical laboratory values. The sample size of 40 in the control group is somewhat small and may well be a limitation of our study. On the other hand, a particular strength of this study is the special efforts made to ensure the homogeneity of the specimens (including the control group) by collecting them precisely on day LH+7, which would have reduced the variance of results.

Our finding agrees with that of Achilles et al. (26), who found that plasma cells were commonly present in the endometrium of asymptomatic, fertile, and healthy women and that their presence alone, in small numbers, may not signify upper genital tract inflammation. Together, these studies suggest that the hitherto held view that the finding of one or more plasma cells in the endometrium is abnormal and diagnostic of CE may lead to overdiagnosis of the condition. Some

previous studies used hysterectomy specimens as control (21, 22), which calls into question their validity because many of those subjects could well have had uterine pathology.

Prevalence of CE in Women with Reproductive Failure

Using the reference range established and based on the CD138+ cell density quantification method, the prevalences of CE in the three subgroups of women with reproductive failure (10.8% for women with RM, 7.7% in women with RIF, and 10.4% in women with infertility) were not significantly different from that of the fertile group. In addition, the prevalence of CE among the entire group of reproductive failure (18/180, 10.0%) was also not significantly different from the fertile group. This finding agrees with the reports by Kasius et al. (27, 28) who observed in a randomized controlled trial that the prevalence of CE in a population of symptomatic infertile patients was low (2.8%) and that the contribution of CE to reproductive failure could have been overestimated in earlier studies. Further analysis of data in Table 2 showed that the use of the cell count per randomly chosen HPF quantification method or the cell count per section quantification method both resulted in overestimation of prevalence rates. Moreover, the application of arbitrarily chosen diagnostic criteria used in the literature also led to overestimation of the prevalence rates. Our observation provides an explanation for the rather high prevalence rates of CE (up to 60%) reported in earlier studies (Table 1).

Precise Timing of Specimen

A notable feature of our study was that we obtained all our specimens on a precise chronologic date, that is exactly 7 days after the LH surge. Although is considered to be acceptable to have specimens collected on LH+7 $\pm$ 1 day, we preferred to include only samples on a precise chronologic date to reduce possible variance in results due to cyclic changes in the cell count. Several studies have suggested that the prevalence of CE was higher when the biopsy was obtained in the proliferative phase than in the secretory phase (29–31). It remains possible that timing of the biopsy in different stages of the cycle could be a confounding variable.

Other Diagnostic Method

In addition to the identification of plasma cells in endometrial biopsy specimens, hysteroscopy has been proposed as an alternate method of diagnosis for CE (32–34). Although hysteroscopy has an accuracy rate of 92.7% in the diagnosis of CE (35), histologic identification of plasma cells remains the criterion standard of diagnosis of CE (12, 16, 17).

Although it is thought that CE is due to an underlying infection, routine microbial culture of endometrial secretion in women with CE is often negative and so precludes its use in clinical diagnosis (36). However, it is now possible to examine the entire microflora present in the endometrium with the use of genomic testing (microbiome study). A recent study by Moreno et al. (37) demonstrated the existence of an endometrial microbiota that is highly stable around the time of implantation and that changes in microbiota profile appeared to be associated with adverse reproductive outcomes. That finding adds a novel microbiologic dimension to our understanding of CE. It would be of interest to establish what specific changes in microbiome induce the emergence of plasma cells in the endometrium.

Consensus and Clinical Significance

A review of the literature on the prevalence of CE revealed that many investigators used different methods of quantification and applied different diagnostic criteria (Table 1), often without justification. There was a lack of consensus in the diagnostic approach to define CE. To make progress in the field and to provide effective and appropriate treatment to women with reproductive failure, a consensus on the quantification method and diagnostic criteria is essential. We hope that the findings in the present study serve to highlight the importance of such a development. Although we have put forward data and argument to support that "plasma cell density" measurement is a more reliable method of plasma cell assessment for the diagnosis of CE, the proof of such a concept requires clinical studies to confirm that the measurement is of useful prognostic value and leads to effective treatment.

In conclusion, we have found that because plasma cells may be present in small number in the endometrium of fertile subjects, the quantification of plasma cell density improves the accuracy of the diagnosis of CE, and that the prevalence of CE in women with reproductive failure was only 10%, which is lower than previously reported.

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